

IS INCREASED NORMAL WHITE MATTER GLUTAMATE CONCENTRATIONS A PRECURSOR OF GLIOSIS AND DISEASE PROGRESSION IN MULTIPLE SCLEROSIS?

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Introduction:

The multiple sclerosis (MS) severity scale (MSSS) is a new scoring procedure to clinically characterize the rate of disease progression in MS, rather than the disability of the patient [1]. The latter is often characterized using the expanded disability status score (EDSS) [2]. The progress rate of the disease, magnetic resonance imaging (MRI)-based measures of 'black hole lesions', and atrophy have all been shown to be predicted well by MSSS [3,4].

Proton (¹H) Magnetic Resonance Spectroscopy (MRS) has been used to characterize brain metabolites non-invasively during disease progression in MS. The interpretation of ¹H MRS results from the diseased MS-brain varies widely in the literature, typically a result of small patient and control subject cohorts, and also of the unfortunate and widespread usage of metabolite ratios in the analyses, obtained using a wide range of data acquisition parameters. By using a metabolite ratio, one obviously cannot determine which metabolite in the ratio has changed, unless there is a known specific metabolic relation between the metabolites.

The most common observation in MS-patients, using absolutely quantified ¹H MRS of normal appearing white matter (NAWM) in MS, is a stable, or slightly decreased, N-acetyl aspartate (NAA) concentration [5], a metabolite which is generally believed to be a neuron specific metabolite [6], or a marker. Other common observations are increases in *myo*-Inositol (*myo*-Ins), which is a glia specific marker [7], which is involved in the membrane phospholipids breakdown [8], and also an increase in the creatine/phosphocreatine (Cr) concentrations [5]. The latter compounds (Cr, or tCr) are much more concentrated in glial cells, than in neurons [7], and therefore are indicators suggestive of such cells. The glutamine (Gln) and glutamate (Glu) metabolism has recently been identified to be an important factor in demyelinating diseases of the central nervous system (CNS). Excessive extracellular concentration of Glu has been shown to induce apoptosis, or necrosis, of the oligodendrocytes, which are the myelinating cells of the CNS [9]. Recently a significant increase of Glu in NAWM, in combination with a non-significant increase of Gln was found in MS-patients using a TE-varied PRESS technique at 3 T [10]. Using short echo time single voxel spectroscopy (SVS) at 1.5 T, the detection of Glu and Gln is more difficult but possible with reasonable certainty [11].

Purpose:

Our aims were to quantitatively investigate the metabolite concentrations in normal appearing white matter (NAWM) in MS-patients, and also to investigate possible correlations between EDSS and MSSS and metabolite concentrations. To minimize the interference from lesion contamination in the MRS measurement, a refined novel analysis procedure had to be developed in order to correct for partial volume effects in tissues near plaques.

Materials and Methods:

108 examinations from 48 patients with Clinically Definite MS (CDMS) were included retrospectively from several MRS studies. T₁, T₂, and proton density MRI, and four white matter ¹H MRS single voxel PRESS (Point-Resolved Spectroscopy) spectra were acquired in each subject using echo time 35 ms and repetition time 6000 ms on a 1.5 T MR-scanner, see [12] for detailed description. Absolutely quantified NAWM metabolite concentrations were determined using a mixed linear model (MLM) analysis that included the degree of T₂ lesion contamination in each voxel visually estimated by a radiologist. The T₂ lesion contamination of the MRS voxels was also used as an estimate of 'lesion load' at each exam. The corrected metabolite concentrations were then correlated with clinical measures of the patients' status, including EDSS and MSSS.

Results:

The axonal marker NAA concentration did not correlate with either EDSS or MSSS, nor did the metabolite ratios NAA/water and NAA/Cr. The glial cell markers Cr and *myo*-Ins correlated positively with EDSS. Cr and Glu correlated positively with MSSS as well as the metabolite ratios Cr/W, Glu/W and Glu/NAA. The 'estimated lesion load' correlated positively not only with EDSS, but also with the number of bouts since disease onset. Importantly, it did not show strong correlation with MSSS. In Fig 1. scatter plots and significance levels of the observed correlations are shown. The correlations Cr and Glu versus MSSS were also confirmed in an analysis excluding all voxels with more than 5% T₂ lesion contamination.

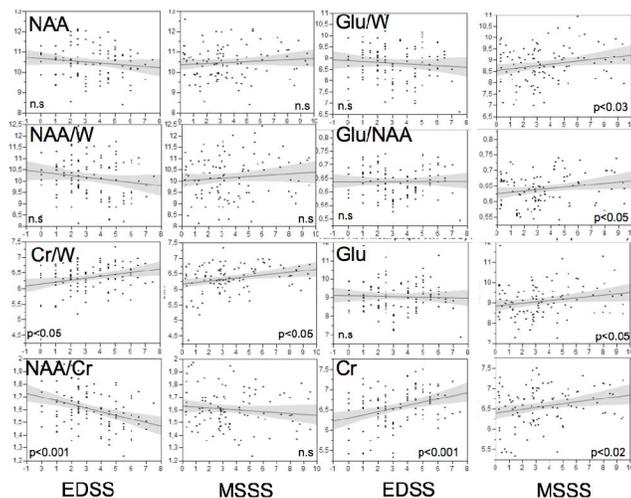


Fig 1. Plots showing fitted regression slope and confidence interval for estimated correlations of NAA, NAA/W, Cr/W, NAA/Cr, Glu/W, Glu/NAA, Glu, and Cr to EDSS and MSSS. Note that Cr/W, Glu/W, and Glu/NAA all showed significant positive correlation to MSSS, as did Cr and Glu, indicating robustness of the results against scaling errors in the absolute quantification procedures. W is the T₂-relaxation-corrected water concentration. All metabolites, including water, were plaque-corrected prior to this analysis, which includes all examinations of the MS-patients.

Discussion and Conclusion:

The most interesting findings were the unchanged concentrations of NAA, and the concomitant increase of Cr and *myo*-Ins during the course of disease progression in MS-patients. These not only indicated a constant axonal density, but also that a simultaneous development of gliosis occurred.

These processes are most likely linked to demyelination, as well as development of white matter atrophy, a process in which the demyelinated volume is replaced by the surrounding tissue leading to a net loss of white matter according to theory developed in [13]. As a consequence of this process, axons in NAWM are probably damaged, which leads to a higher concentration of glia cells relative to the axonal volume.

The positive correlation that was found between MSSS, and the Glu and Cr concentrations in NAWM, in combination with a complete lack of correlation between lesion load and MSSS, suggests that altered glutamate metabolism, and subsequent demyelination and gliosis, is an important pathophysiological mechanism in MS.

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